PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NOTA/P30447PC	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/002569	International filing date *(day/month/year)* 16.06.2004	Priority date *(day/month/year)* 19.06.2003
International Patent Classification (IPC) or national classification and IPC C07K5/062, C07K5/065, C07K5/068, C07K5/072, A61P7/02, A61K38/05		
Applicant THE NOTTINGHAM TRENT UNIVERSITY		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 12 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand 18.04.2005	Date of completion of this report 16.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Fuhr, C Telephone No. +31 70 340-3510

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/002569

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-83	as originally filed
Claims, Numbers	
1-44	received on 18.04.2005 with letter of 18.04.2005
Drawings, Sheets	
1/30-30/30	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 45-62
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/002569

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-44
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-44
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-44
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/GB2004/002569

1 Re Item I
Basis of the report

The amended set of claims is acceptable according to Article 34(2)(b) PCT.

2 Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:
D1: D.H. Pliura et al. 'Irreversible Inhibition ...', J. Enzyme Inhibition 6, p. 181-194
D2: EP411895

2.1 Novelty (Article 33(2) PCT)

2.1.1 The subject matter of claims 1-28 which is related to compounds is novel. Claims covering pharmaceutical compounds comprising the compounds (claim 29), methods for making the compounds (claims 30-33), first or second medical applications of said compounds (claims 34-44) are novel, too.

2.2 Inventive Step (Article 33(3) PCT)

2.2.1 The document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and discloses Cbz-Phe-$NH(CH_2)_nCOCH_2S^+(CH_3)_2$ with n= 1-5 and their acitivity in inhibiting transglutaminases (see table 1 on page 187).
The subject-matter of claim 1 therefore differs from this known D1 in that the compounds of application have a carboxylic group bound to the α-C-atom of the ω-aminoalkyl-keto-moiety. The compounds of claim 1 have the same activity in inhibiting transglutaminases as the compounds disclosed in D1.
The problem to be solved by the present invention may therefore be regarded as provision of further compounds which have transglutaminase inhibiting activity.
Part of the subject matter of claim 1 further differs from D1 in that the compounds

have a thio-imidazolium salt in position R2 of formula I. This modification is described also, see D2, examples I-III. The compounds also have transglutaminase inhibiting activity page 2, lines 39-44). Again it can be expected that the substitution of the dialkylthiogroup in R2 by the thio-imidazolium group will result in further compounds having transglutaminase activity.
The solution of the problem underlying the invention does comprise an inventive step (Article 33(3) PCT) because the compounds of claim 1 have a higher solubility in water than the compounds of D1. In fact D1 states that stock solutions contained the transglutaminase inhibitor in dimethylsulfoxid (DMSO), and it also discloses that the in vitro transglutaminase activity assays were performed in buffers containing DMSO (page 183, lines 5-7 of first paragraph, line 4 of second paragraph, lines 5-8 of third paragraph). In contrast, the compounds of present claim 1 are highly soluble in water. Example 2 on page 65 of the description teaches, that stock solutions were prepared in water (lines 21-23). This allows the execution of in vivo test as described in example 4 of the application.

2.2.2 The dependent claims 2-28 are inventive as well (Article 33(3) PCT).

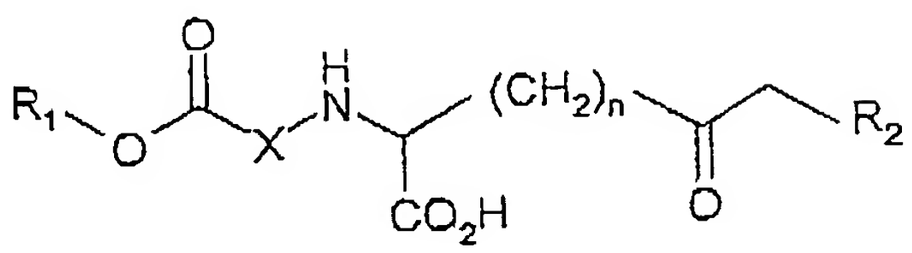
2.2.3 The subject matter of claims 29-44 includes pharmaceutical formulations (claim 29) comprising the compounds of claims 1-28, methods of production (claims 30-33) of said compounds and first (claim 34) and second (claims 35-44) medical uses of said compounds. The inventive step inherent to these claims is dependent on the inventive step assessment of the compounds they relate to or employ. Because the compounds of claims 1-28 are inventive, the subject matter of claims 29-44 is inventive, too.

2.3 Industrial applicability (Article 33(4) PCT)

2.3.1 The subject matter of claims 1-44 is industrial applicable (Article 33(4) PCT).

CLAIMS

1. A compound having the following formula I:



I

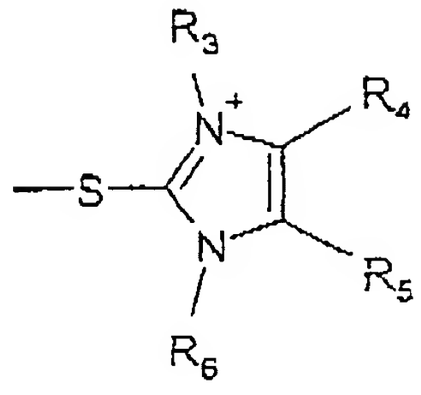
wherein:

'X' represents an α-amino acid group wherein the α-amino group of the amino acid is bound to the R_1-O-CO- group and the carboxy group of the amino acid is bound to the R_2-CH_2-CO-$(CH_2)_n$-CH(CO_2H)-NH- group;

'n' is an integer between 1 and 4;

'R_1' represents benzyl, t-butyl or 9-fluorenylmethyl; and

'R_2' represents



wherein R_3, R_4, R_5 and R_6 each independently represent lower alkyl

or $-S^+R_7R_8$, wherein R_7 and R_8 each independently represent lower alkyl

or a pharmaceutically and/or veterinarily acceptable derivative thereof.

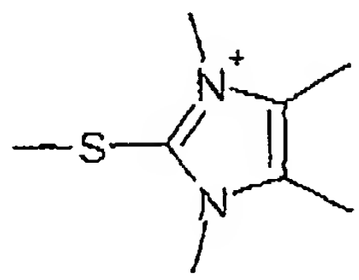
2. A compound according to Claim 1 wherein X is an *L*-amino acid group.

3. A compound according to Claim 1 or 2 wherein X is selected from the group consisting of phenylalanine, glutamine (or an N-substituted derivative thereof), isoleucine, alanine, glycine, tyrosine, proline, serine, lysine and glutamic acid.

4. A compound according to any one of the preceding claims wherein 'n' is 2.

5. A compound according to any one of the preceding claims wherein R_1 is benzyl.

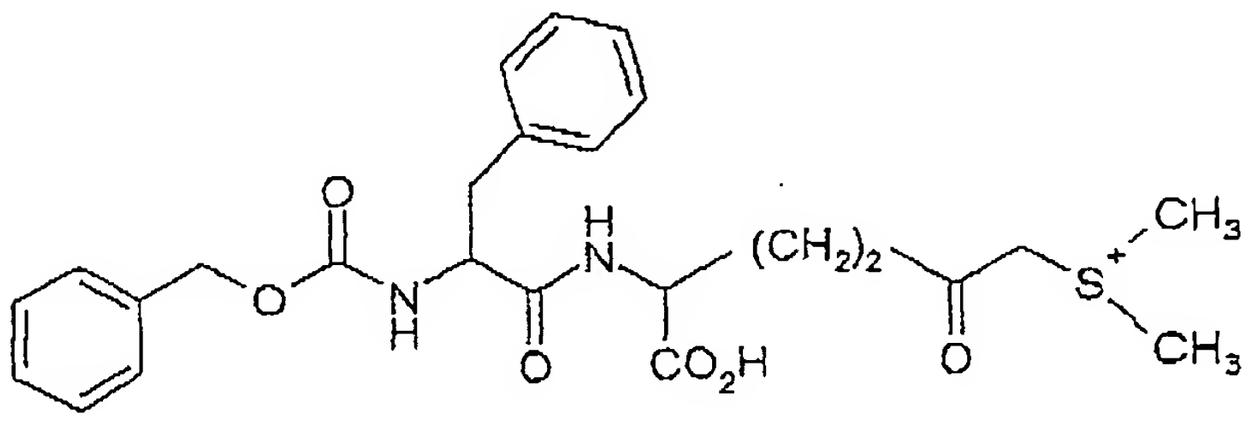
6. A compound according to any one of the preceding claims wherein R_2 represents



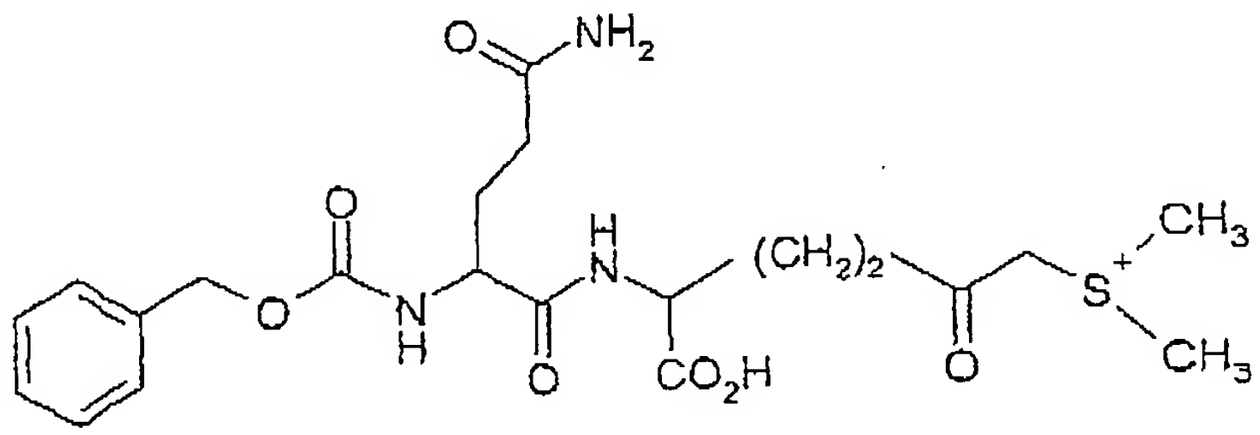
7. A compounds according to any one of the preceding claims wherein R_2 represents $-S^+R_7R_8$, wherein R_7 and R_8 each independently represent lower alkyl.

8. A compound according to any one of the preceding claims wherein R_3, R_4, R_5, R_6, R_7 and/or R_8 are $-CH_3$ or $-CHCH_2$.

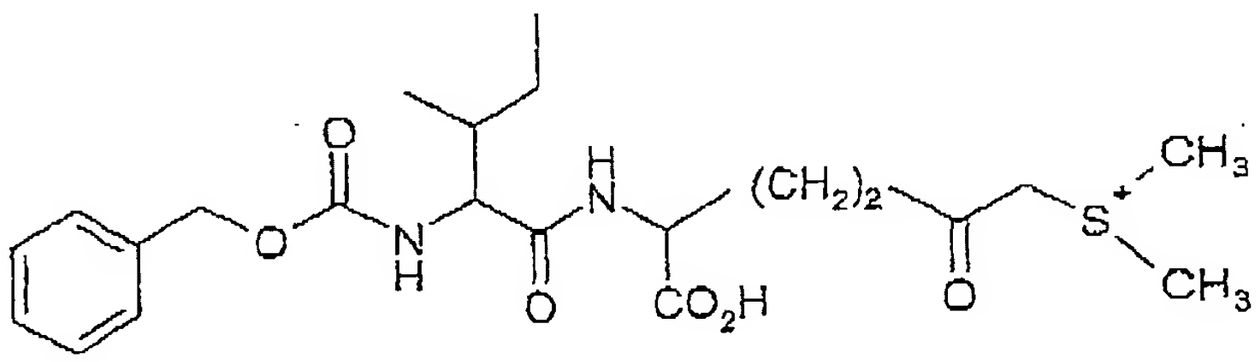
9. A compound according to Claim 1 having the following formula:



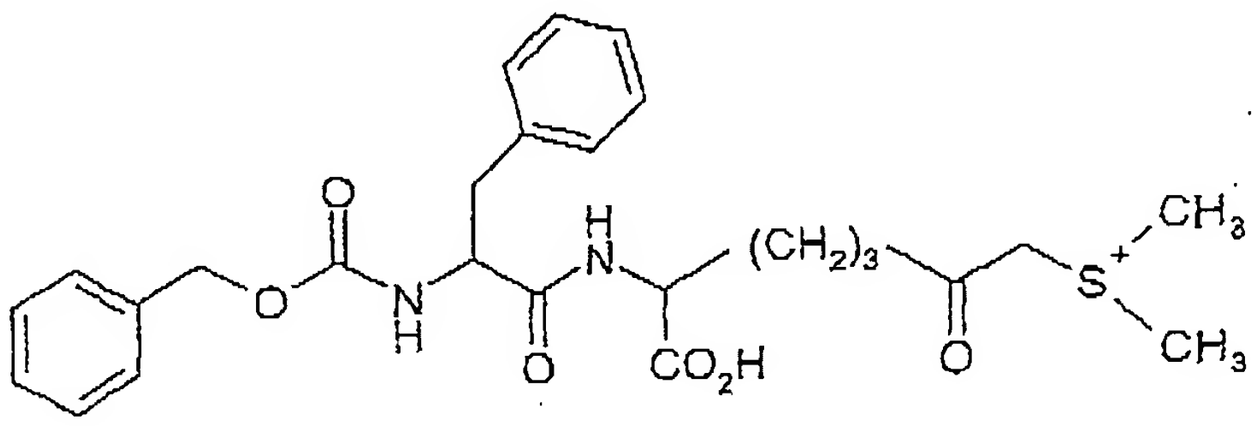
10. A compound according to Claim 1 having the following formula:



11. A compound according to Claim 1 having the following formula:

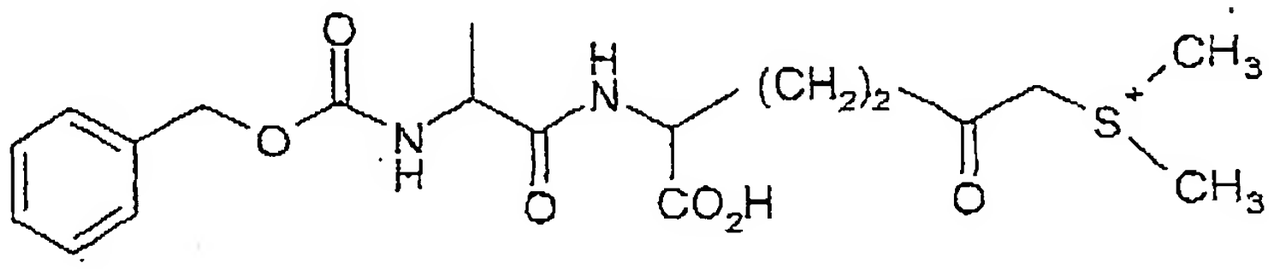


12. A compound according to Claim 1 having the following formula:

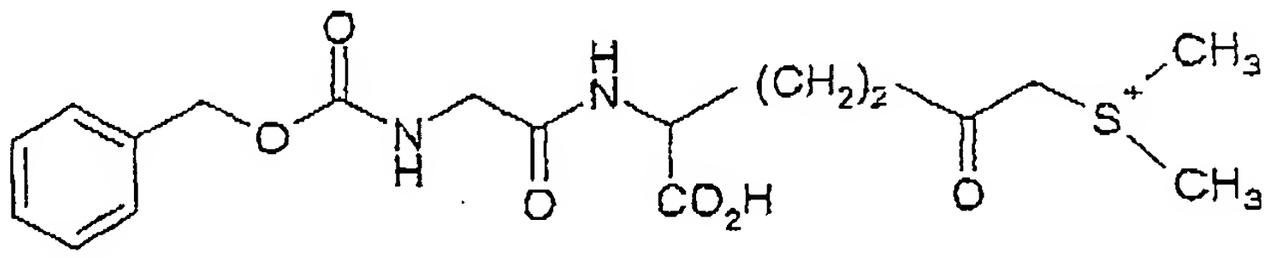


13. A compound according to Claim 1 having the following formula:

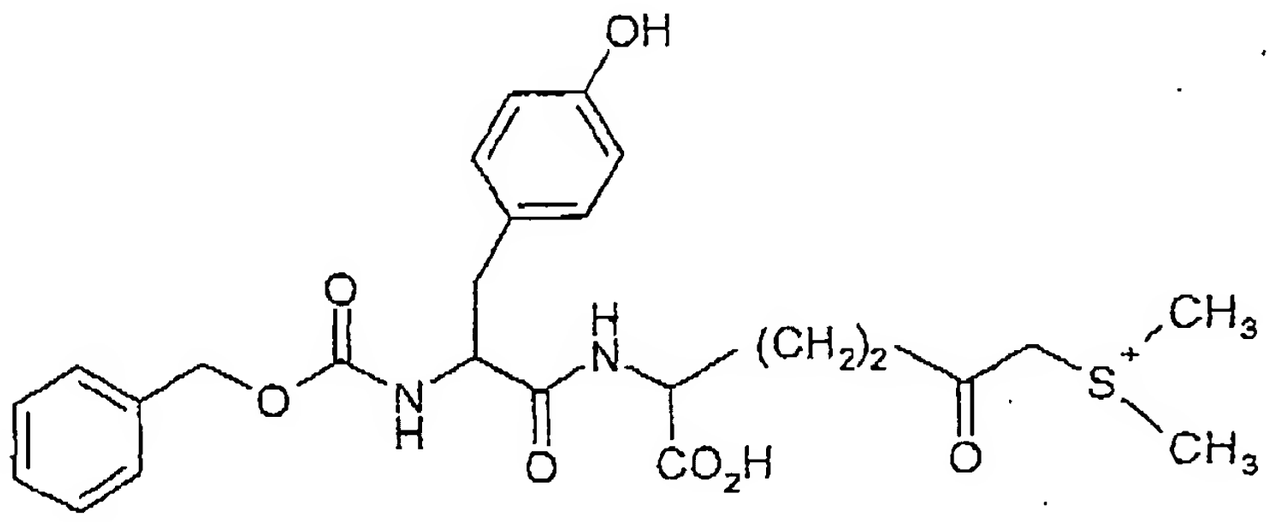
14. A compound according to Claim 1 having the following formula:



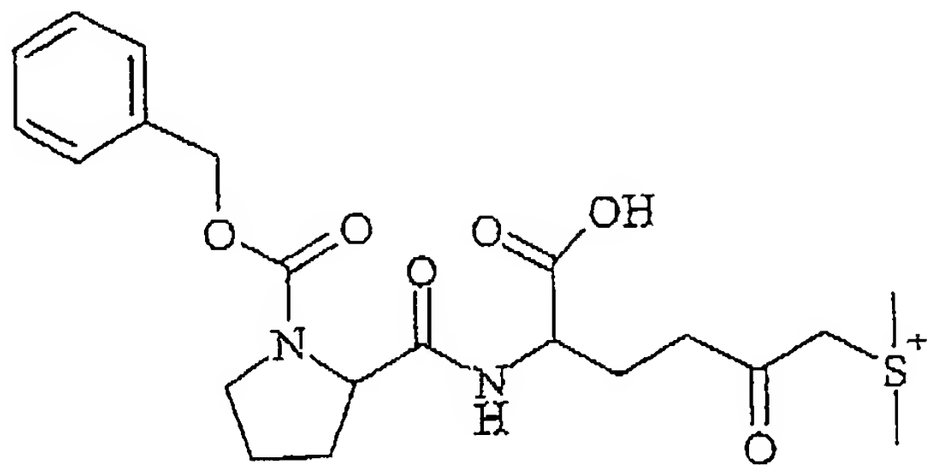
15. A compound according to Claim 1 having the following formula:



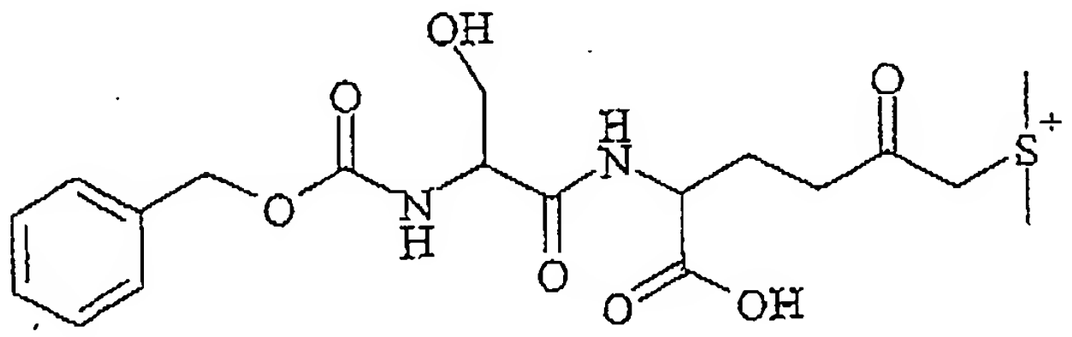
16. A compound according to Claim 1 having the following formula:



17. A compound according to Claim 1 having the following formula:

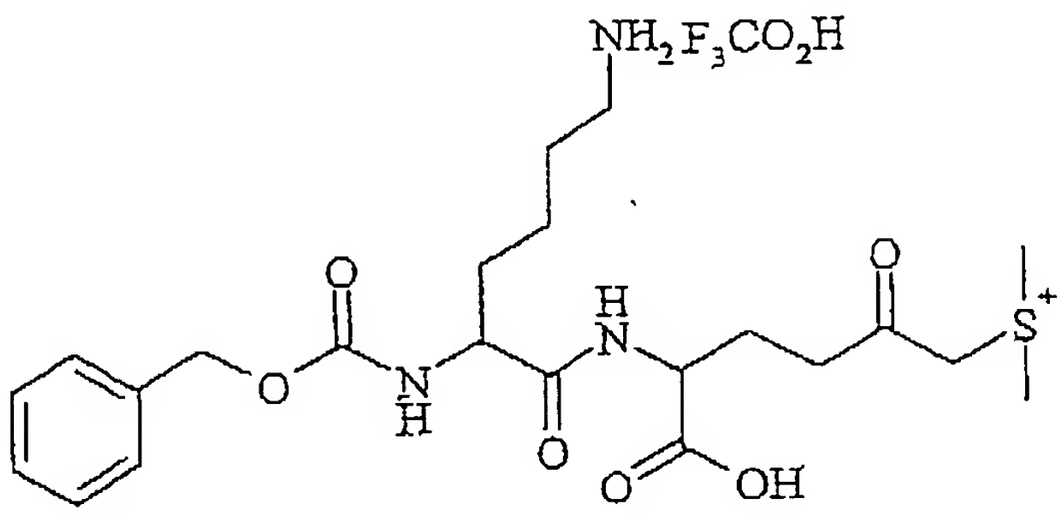


18. A compound according to Claim 1 having the following formula:

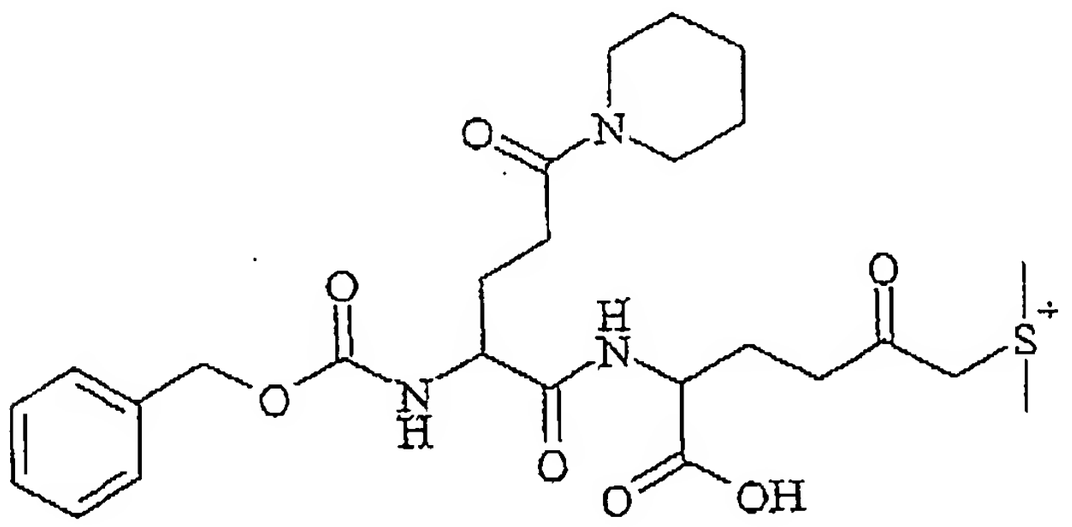


19. A compound according to Claim 1 having the following formula:

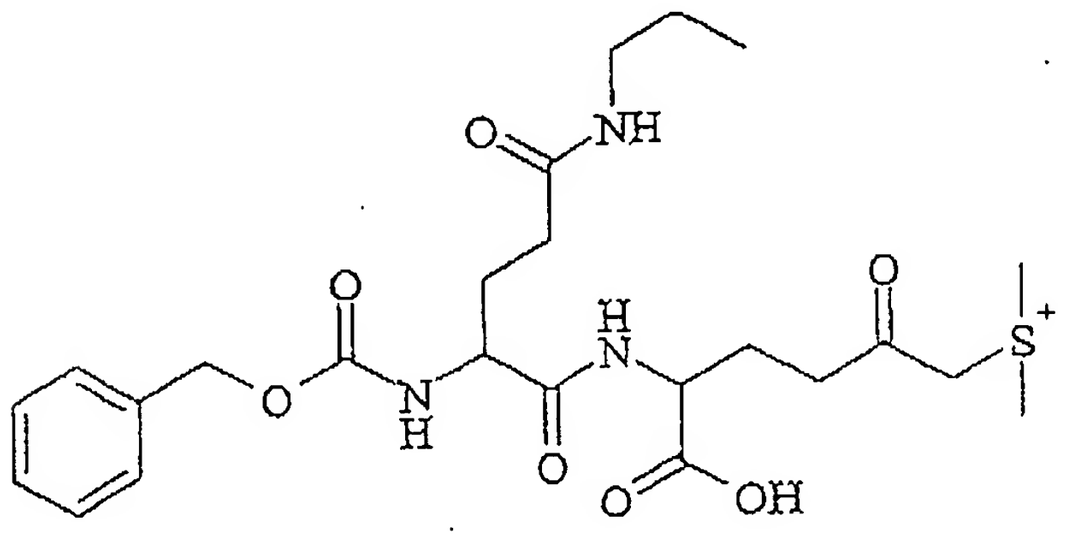
20. A compound according to Claim 1 having the following formula:



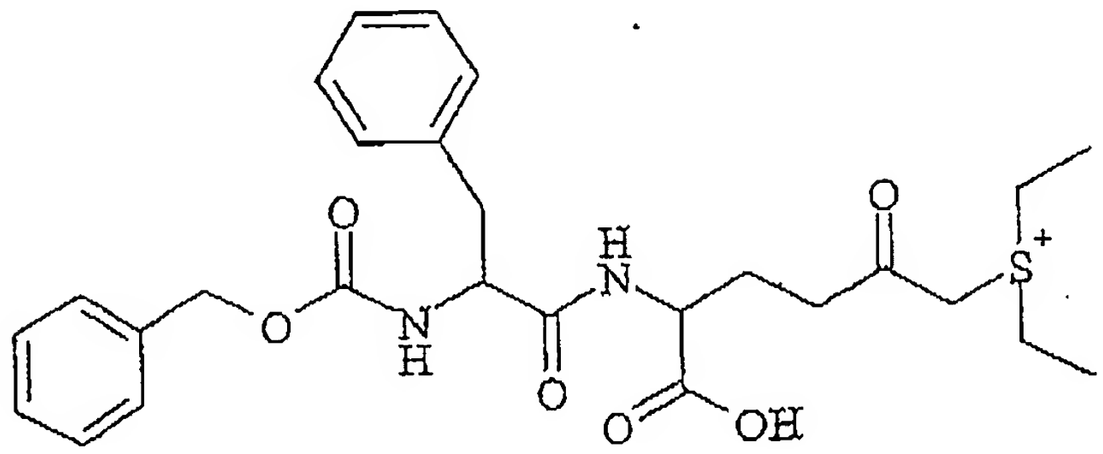
21. A compound according to Claim 1 having the following formula:



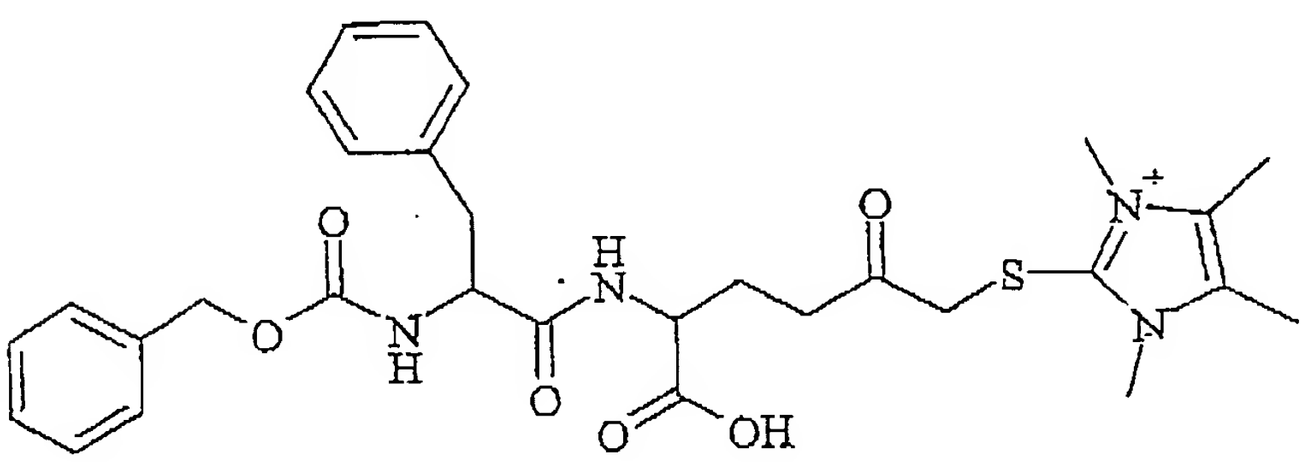
22. A compound according to Claim 1 having the following formula:



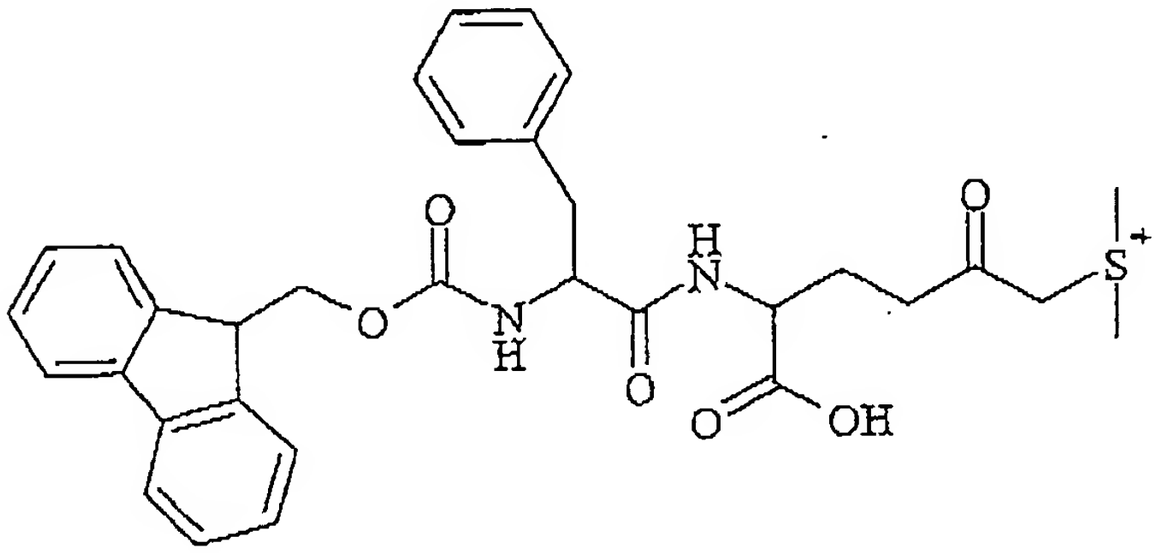
23. A compound according to Claim 1 having the following formula:



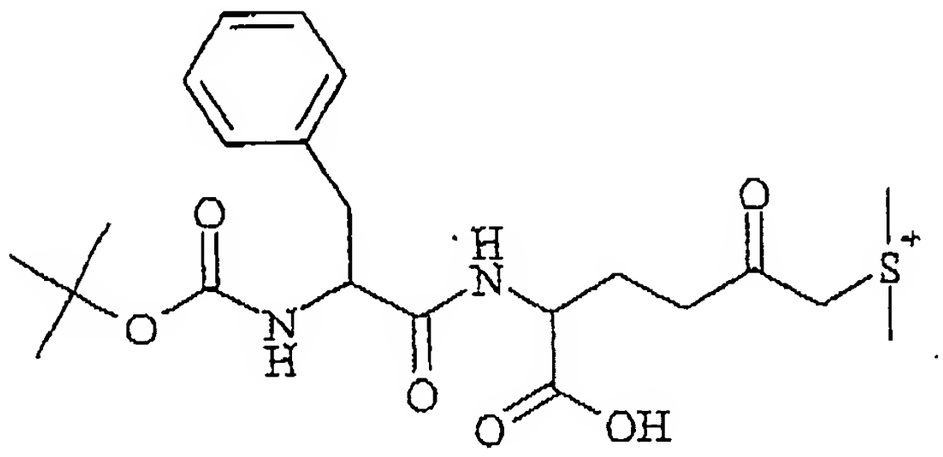
24. A compound according to Claim 1 having the following formula:



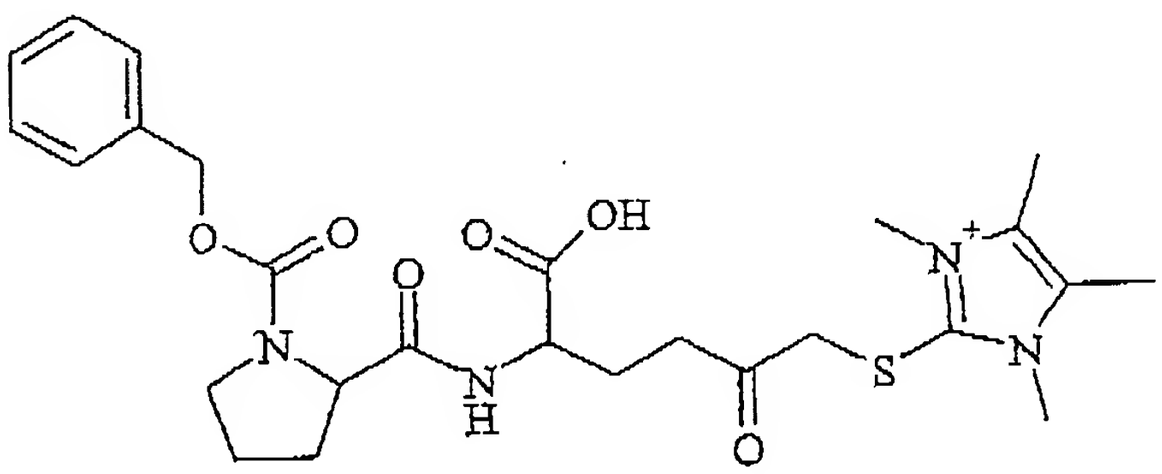
25. A compound according to Claim 1 having the following formula:



26. A compound according to Claim 1 having the following formula:



27. A compound according to Claim 1 having the following formula:



28. A compound according to any one of Claims 1 to 27 in the form of a bromide salt.

29. A pharmaceutical formulation comprising a compound according to any one of Claims 1 to 28 and a pharmaceutically acceptable carrier.

30. A method for making a compound according to any one of Claims 1 to 28 comprising the following steps:

(a) reacting an *N*-α-protected (*e.g.* CBZ, FMOC or BOC protected) amino acid *N*-hydroxy-succinimide or *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine, and treating the resulting coupled product with hydrogen bromide; and

(b) reacting the bromomethyl ketone produced in step (a) with dimethyl sulphide, diethyl sulphide or 1,3,4,5-tetra-methyl mercapto-imidazoline-2-thione.

31. A method according to Claim 30 wherein the *N*-α-protected amino acid *N*-hydroxysuccinimide ester is CBZ, FMOC or BOC protected.

32. A method according to Claim 30 or 31 wherein step (a) comprises reacting an *N*-α-protected amino acid *N*-hydroxy-succinimide or *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine in the presence of tetrahydrofuran (THF), water and triethylamine followed by reacting the products thereof with hydrogen bromide in the presence of ethyl acetate.

33. A method according to Claim 31 or 32 wherein the *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide ester is selected from the group consisting of *N*-α-CBZ-*L*-phenylalanine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glutamine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-isoleucine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-alaninal *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glycine

N-hydroxysuccinimide ester, *N*-α-CBZ-*L*-proline *N*-hydroxysuccinimide ester, *N*-α-CBZ-*L*-serine *N*-hydroxysuccinimide ester, *N*-α-CBZ-*L*-tyrosine *N*-hydroxysuccinimide ester, *N*-α-CBZ-*L*-glutamic acid *N*-hydroxysuccinimide ester, *N*-α-CBZ-*L*-lysine *N*-hydroxysuccinimide ester and *N*-α-CBZ-*L*-tyrosine *para*-nitrophenyl ester.

34. A compound according to any one of Claims 1 to 28 for use in medicine.

35. Use of a compound according to any one of Claims 1 to 28 in the preparation of a medicament for inhibiting a transglutaminase

36. The use according to Claim 35 wherein the transglutaminase is a tissue transglutaminase.

37. The use according to Claim 35 or 36 wherein the medicament is for treating a disease/disorder selected from the group consisting of fibrosis, scarring, neurodegenerative diseases, autoimmune diseases, thrombosis, proliferative disorders, AIDS, psoriasis and inflammation (such as chronic inflammatory diseases).

38. The use according to any one of Claims 35 to 37 wherein the medicament is for treating cancer.

39. The use according to any one of Claims 35 to 37 wherein the medicament is for treating fibrosis and/or scarring.

40. The use according to Claim 39 wherein the medicament is for treating renal scarring.

41. Use of a compound according to any one of Claims 1 to 28 in the preparation of a medicament for preventing or treating rejection of a transplanted organ.

42. A use according to Claim 41 wherein the organ is a heart, lung, kidney or liver.

43. A use according to Claim 41 or 42 wherein the organ is treated prior to transplantation.

44. A use according to any one of Claims 41 to 43 wherein the organ is treated during and/or after transplantation into a patient.